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GAMETOGENIC CYCLE IN *VERTIGO PUSILLA* O. F. MÜLLER, 1774 (GASTROPODA: PULMONATA: VERTIGINIDAE)

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ABSTRACT: Gonads of *V. pusilla* at various growth stages were examined with standard histological methods. The first gonial cells appeared at the stage of 3.3 whorls: few and poorly developed gonad vesicles contained fine mitotically dividing cells and cells in meiotic prophase, with the first growing oocytes in the peripheral parts of the vesicles. At 4-whorl stage the oocytes entered vitellogenesis and increased in volume. In subadult individuals the oocyte volume and the gonad volume increased considerably; besides the advanced vitellogenic oocytes new oocytes appeared, the number of spermatids increased and the first spermatozoa were observed. The gonad of pre-reproductive adults contained numerous late-vitellogenic oocytes, young growing oocytes and many spermatozoa. In post-reproductive individuals there were no mature oocytes, spermatozoa were few, but the presence of young oocytes and mitotically dividing cells indicated resumption of gamete production.

KEY WORDS: terrestrial snails, Pulmonata, *Vertigo pusilla*, gonad, gametogenesis

INTRODUCTION

Pulmonate snails display a great variety of life cycles. The diversity involves nearly all life cycle components, the most important being longevity, fertility, rate of growth and maturation, courtship behaviour, mode of reproduction (bi- or uniparental, in the latter case self-fertilisation or parthenogenesis). This results in an equally great variety of evolutionary strategies (FRETTER & GRAHAM 1983, TOMPA 1984, HELLER 1990). Being hermaphrodites, pulmonates represent a different evolutionary quality compared to animals with separate sexes. Despite their obvious advantages as objects of studies on the evolution of life cycles, reasonably complete life history information exists only for a small fraction of species (for a recent review see MALTZ 2003b). Even less is known about processes that take place in the pulmonate gonad – the data are limited to rather superficial descriptions of gametogenesis in adult specimens of a few species (PARIVAR 1978, CSABA & BIERBAUER 1979, HELLER et al. 1997, KORALEWSKA-BATURA 1994), or purely morphological descriptions of the development of the reproductive system (ENÉE & GRIFFOND 1983, JACKIEWICZ & ZBORALSKA 1994) while ontogenetic development of the hermaphrodite gland has been studied in one species only (MALTZ 2003a).

Because of the peculiarities of life cycles of members of *Vertigo* (POKRYSZKO 1990a, b, 2003; CAMERON 2003, KILLEEN in press), and the widespread aphallism (WATSON 1923, POKRYSZKO 1987, 1990a), histological analysis of their gonad may contribute to understanding of many problems, for example regulation of the onset of breeding or the mechanism of uniparental reproduction. In spite of this there have been no such studies to date. The aim of this paper was a preliminary description of changes which take place in the gonad of *Vertigo pusilla* O. F. Müller, 1774 in relation to ontogeny and phase of reproductive activity.

MATERIAL AND METHODS

The material included 33 immature and mature individuals of *V. pusilla*, collected in a mixed deciduous-coniferous forest near Bardo Śląskie (S.W. Poland) (Table 1). Some of the individuals collected in June 2002 were then kept in the laboratory in order to obtain consecutive growth stages (for details of laboratory culture see POKRYSZKO 1990b). Since, like in all members of the genus, completion of apertural barriers in *V. pusilla* amounts to attainment of sexual maturity, it is possible to unequivocally distinguish between mature and immature snails.

The snails collected in July 2001 were post-reproductive individuals. The adults from the laboratory culture (see Table 1: June 2002) were dissected before reproduction.

The material was dissected under the stereomicroscope, in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4). The shell was removed with forceps. Gonads were removed from adult individuals; primordial gonads of immature specimens were removed together with the adjoining digestive gland tissue (subadults) or even (stages up to 4 whorls in which gonad could not be located upon dissection) all the apical part of the visceral sac was removed. Specimens of 4 or more whorls were checked for aphallic/euphallic condition. All the dissected individuals at these stages were aphallic.

The material, preserved in glutaraldehyde, was repeatedly rinsed with phosphate buffer and postfixed in 1% osmium tetroxide. Following dehydration in acetone series, it was embedded in epoxy resin Epon 812, cut into semithin sections, stained with 1% methylene blue in 1% borax, and examined and photographed in light microscope Olympus BHS. Because of the difficulties implied in preserving the material, photographic documentation was made for the following stages only: 3.3 whorls, subadult specimens, pre-reproductive adults, post-reproductive adults.

Table 1. List of the material examined

Growth stage	Number of individuals	Date of collection
< 2 whorls	1	June 2002
2–3 whorls	9	June 2002
3.1–4 whorls	7	June 2002
4.1–5 whorls (subadult)	3	June 2002
Adult	5	July 2001
Adult	5	June 2002
Senile	3	June 2002

RESULTS

The following parameters were taken into account when analysing the gonad: size, advancement of development (presence and number of gonad vesicles), number and kind of gonial cells visible on the slides: oocytes at various stages of oogenesis (small previtellognic and vitellogenic oocytes), mitotically dividing cells, cells in meiotic prophase, spermatogonia, spermatids and packets of spermatozoa. In individuals of 2 or fewer whorls, gonad primordia were practically inisible and no gonial cells could be observed on slides.

The first gonial cells appeared in individuals of 3.1–4 whorls. At the stage of 3.3 whorls the gonad vesicles were few and small; they contained fine, mitotically dividing cells and cells in meiotic prophase. The first growing oocytes appeared in the peripheral portions of the vesicles, in direct contact with the gonad wall (Figs 1, 2, 3). Their nuclei were large, with well-developed nucleoli (usually two), and centrally located (Figs 2, 7). Cells in meiotic prophase were located mainly in the central parts of sections through the gonad vesicles (not shown). Somatic cells, visible on the surface of oocytes, stained stronger with methylene blue. The gonad vesicles were separated from the digestive gland tissue by a pigmented epithelium (Fig. 1).

At the stage of 4 whorls the oocytes considerably increased in size, and the first yolk spheres and lipid droplets appeared in their cytoplasm. At this stage the oocytes were accompanied by few cells in meiotic prophase (not shown).

In subadult individuals the volume of individual oocytes (filled with yolk and lipids), as well as that of the whole gonad, increased considerably. Besides the oocytes at the stage of advanced vitellogenesis, also young, growing oocytes were present (Figs 4, 5). Cells in meiotic prophase were few, while the number of spermatids increased and the first spermatozoa appeared (Fig. 5). Nuclei of late-vitellogenic oocytes contained each a single, prominent nucleolus (two in growing oocytes) (Fig. 5).

Gonads of pre-reproductive adults were relatively large. Numerous late vitellogenic oocytes filled the lumen of vesicles on transverse sections through the gonad (Fig. 6). In the cortical parts also young, growing ocytes were observed (Figs 6, 7). In the gonad lumen, in the spaces between the vitellogenic oocytes, spermatozoa were present (Fig. 7).

Gonads of post-reproductive adults contained cells immediately after mitotic division (not shown), cells

in meiotic prophase, as well as growing and early vitellogenic oocytes. Mature oocytes were absent, and spermatozoa were very few (Figs 8, 9).

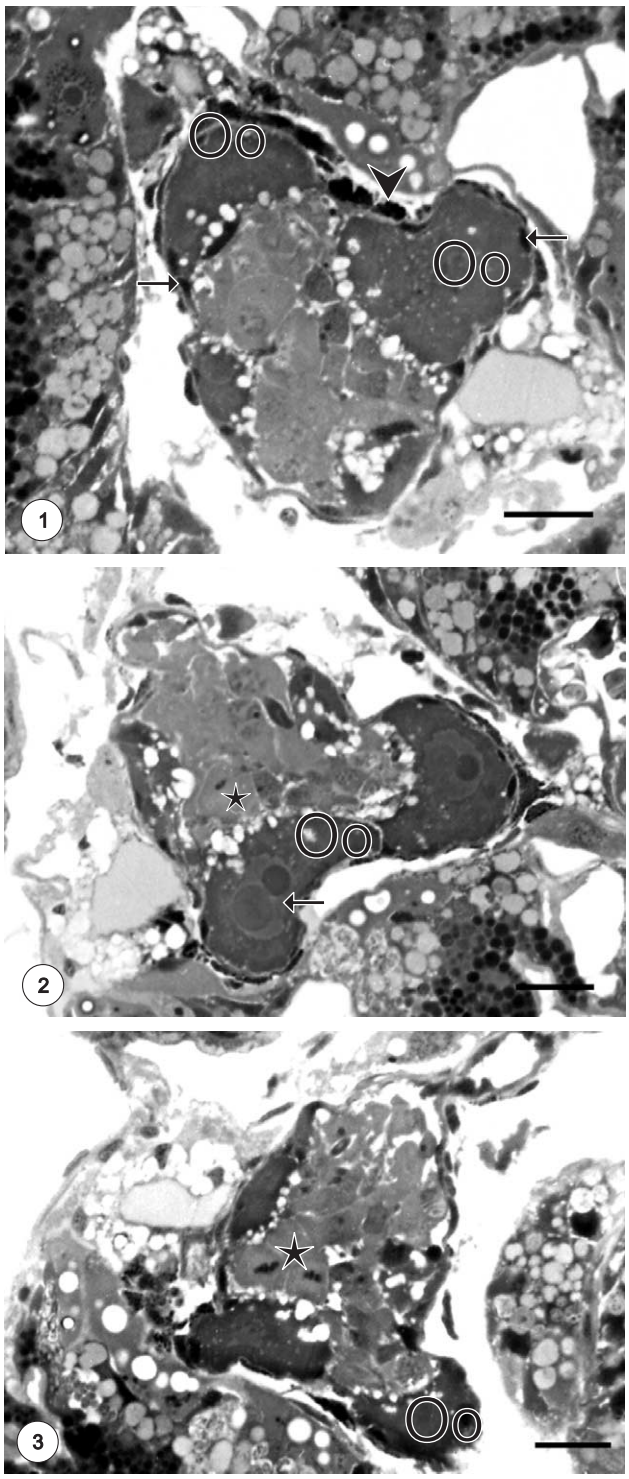
DISCUSSION

Like other pulmonates, *V. pusilla* is hermaphroditic. The structure of its reproductive system, though simplified, conforms to the basic pattern found in most pulmonates (TOMPA 1984, POKRYSZKO 1990a, b). At the same time most (72–95%) individuals in every population are aphallic (POKRYSZKO 1987).

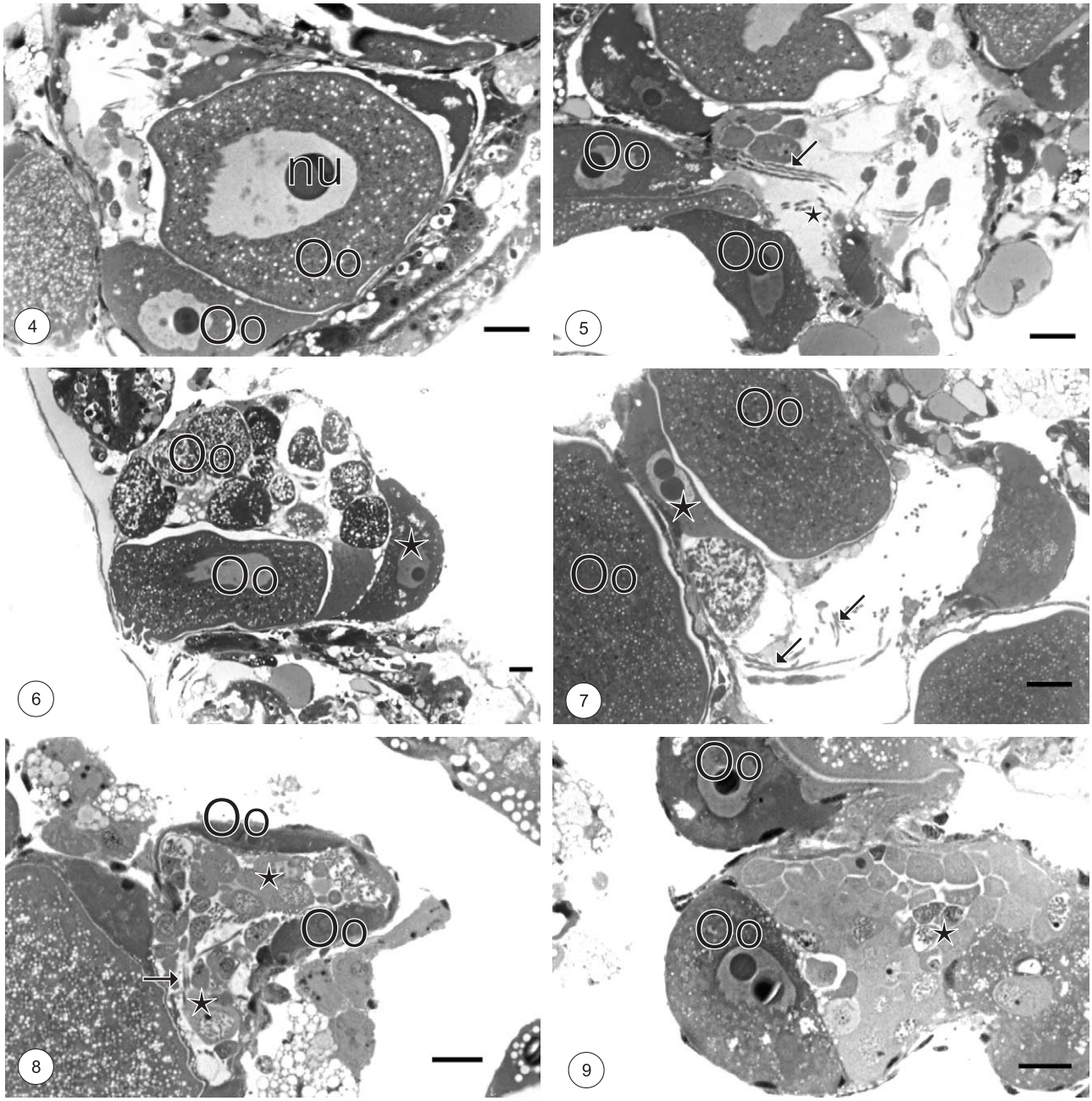
Gonads of the pulmonate snails which have been studied in this respect produce mature gametes of both sexes at the same time (simultaneous hermaphroditism), or spermatozoa are earlier to mature (protandry) (FRETTER & GRAHAM 1964, TOMPA 1984). However, such studies included only species in which there is no aphallism. During copulation in *V. pusilla* aphallic individuals play a role of females, euphallic ones – of males. Both forms are capable of uniparental reproduction but its mechanism (selfing or parthenogenesis) remains unclear (POKRYSZKO 1990b). In this context it is important that the gonad of aphallic individuals is capable of producing gametes of both sexes. The cycle of gamete production in their gonads: continuous growth of oocytes and a short phase of spermatozoa production, does not depart from such cycle in other studied terrestrial pulmonates (MALTZ 2003a, b).

The gonads of pre-reproductive adults contained gametes of both sexes; in post-reproductive adults there were no mature oocytes, and spermatozoa were very few. Considering that the examined adults were all aphallic, that such individuals do not transfer their sperm during copulation and that only very few of them ever mate (POKRYSZKO 1990b), the marked decrease in the number of spermatozoa in post-reproductive adults may indirectly point to selfing as the mechanism of uniparental reproduction.

V. pusilla is short-lived, the lifespan only rarely exceeds one year (POKRYSZKO 1990b). Its reproductive mode is intermediate between semelparity and iteroparity: most individuals reproduce only once in their lifetime, the reproductive season being short and limited to early spring, but few individuals under favourable humidity conditions lay eggs for the second time, in late summer or autumn (POKRYSZKO 1990b). This reproductive mode corresponds with the picture observed in the gonad vesicles. In post-reproductive individuals, though most of them will die soon after reproduction, the gonad resumes its gametogenic cycle, as shown by the presence of numerous mitotic and meiotic divisions and early vitellogenic oocytes, so that repeated reproduction is not exclued.



Figs 1–3. *V. pusilla,* gonads, semithin sections, methylene blue. Scale bars in all figures 0.2 mm.1–3 – 3.3 whorls: 1 & 3 – growing oocytes (Oo) in gonad vesicle, with accompanying somatic cells (arrows) (Fig. 1) and cells in meiotic prophase (asterisks) (Fig. 3); arrowhead indicates pigment (Fig. 1); 2 – in gonad vesicle oocytes (Oo) with nuclei with well-developed nucleoli (arrow) and mitotically dividing cells (asterisk) (Fig. 2)



Figs 4–9. *V. pusilla,* gonads, semithin sections, methylene blue. Scale bars in all figures 0.2 mm. 4 & 5 – subadult: vitellogenic oocyte (Oo) with strongly marked nucleolus (nu) in nucleus (Fig. 4), at this stage few spermatozoa (arrow), spermatids (asterisk) and young, growing oocytes (Oo) are visible in the gonads (Fig. 5); 6 & 7 – pre-reproductive adult: vitellogenic oocytes (Oo), spermatozoa (arrows) and growing oocytes (asterisks); 8 & 9 – post-reproductive adult: cells in meiotic prophase (asterisks), few spermatozoa (arrow), and growing oocytes (Oo)

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